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Newly identified genes linked to endometrial receptiveness: lessons from IVF

“Successful implantation depends on synchronization between the developmental stages of the embryo itself and the complex series of molecular and cellular events induced in the endometrium by paracrine and autocrine regulators during the implantation window.”

A total of 30 years after the first *in vitro* fertilization (IVF) birth in the world, pregnancy and birth rates following IVF attempts remain low. Indeed, two out of three IVF cycles fail to result in pregnancy, and more than 9 out of 10 transferred embryos fail to implant [1]. Although the selection of high-quality embryos and endometrial status assessment have seen important technological progress, implantation rates are still low after controlled ovarian hyperstimulation (COS) for IVF [2]. Implantation failure is thought to result from impairment of embryo development and/or from abnormal uterine receptivity. The endometrium is receptive to blastocyst implantation during a spatially and temporally restricted window, ‘the implantation window’ [3]. In humans, this period begins 6–10 days after the luteinizing hormone (LH) surge and lasts approximately 48 h [4,5].

“Although the selection of high-quality embryos and endometrial status assessment have seen important technological progress, implantation rates are still low after controlled ovarian hyperstimulation for *in vitro* fertilization”

Human endometrial receptivity during natural cycles

Successful implantation depends on synchronization between the developmental stages of the embryo itself and the complex series of molecular and cellular events induced in the endometrium by paracrine and autocrine regulators during the implantation window. This explains that there are many molecular marker candidates to determine the ‘ideal’ endometrium receptive period [6–10]. Several parameters have been suggested for assessing endometrial receptivity, including endometrial

thickness (a classical criterion), endometrial morphology aspect and endometrial and sub-endometrial blood flow using ultrasonography and Doppler (FIGURE 1), noninvasive methods to evaluate uterine receptivity [6,7,9,11]. However, such indirect explorations result in controversy, and their positive predictive value is limited [12–14]. Emerging technology in the assessment of the gametes, competent embryos and endometrial receptivity, the omics allow the identification of biomarkers or to determine the molecular signature of endometrial cells specific to endometrial receptivity. More recently, transcriptomic approaches have been used to identify biomarkers of the human implantation window. Using microarray technology in human biopsy samples, several authors have observed modifications in gene expression profiles associated with the transition of the human endometrium from a prereceptive (LH + 2/4 days) to a receptive (LH + 7/9 days) state (TABLE 1) [15–19]. However, among the various regulated genes, only two genes were in common between all these studies: secreted phosphoprotein (SPP) 1, a glycoprotein involved in cellular adhesion and migration during embryo implantation, and IL15, a progesterone-regulated gene involved in stages immediately before, during and after the apposition step and permitting adequate proliferation of the stroma [20,21]. Disparities between these studies [15–19] may be explained by several factors: type of microarray used, data analysis, statistical methodologies, size of patient samples, ages of subjects and sampling time during the cycle. In addition, only two studies compared the early- and mid-secretory phases in the same patient [16,18], which seems a necessary condition to minimize the impact of interpatient variability. Incorporating these conditions and using a large series of patients (in

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Figure 1. Ultrasonography is useful for evaluating endometrial receptivity.

contradiction to Riesewijk's study), we identified four new biomarkers of endometrial receptivity that were upregulated in the LH + 7 days samples compared with the LH + 2 days samples. These biomarkers are MFAP5, ANGPTL1, EG-VEGF and NLF2, and play a role in the extracellular matrix remodeling of the endothelial cell micro-environment, angiogenesis and the formation of the endothelial fenestration. This group of genes shows potential to be useful prognostic markers of endometrium receptivity (FIGURE 2).

Human endometrial receptivity during COS cycles

COS is a key factor in the success of IVF–embryo transfer (ET). Studies comparing GnRH agonist long protocols with GnRH

Figure 2. Endometrial profiles during the prereceptive and the receptive stages in natural and stimulated cycles. Hierarchical clustering between LH + 2 days and LH + 7 days samples, and between hCG + 2 days and hCG + 5 days samples. Red: upregulated genes; Green: downregulated genes. hCG: human chorionic gonadotropin; PR: Prereceptive; R: Receptive.

antagonist protocols remain controversial, and the impact of these protocols on endometrial receptivity has yielded conflicting results (TABLE 1) [22–26]. Several studies have shown that gene

Table 1. Number of genes significantly modulated in microarray comparison analyses between the early- and mid-secretory stages of the natural and stimulated endometrium cycle.

Study	First sample: (number of samples)	Second sample: (number of samples)	Fold change	Number of genes		Ref.
				Up	Down	
Carson <i>et al.</i> (2002)	LH + 2/4 (3)	LH + 7/9 (3)	≥2	323	370	[15]
Mirkin <i>et al.</i> (2005)	LH + 3 (3)	LH + 8 (5)	≥2	49	58	[17]
Riesewijk <i>et al.</i> (2003)	LH + 2 (5)	LH + 7 (5)	≥3	153	58	[18]
Talbi <i>et al.</i> (2006)	ESP (3)	MSP (8)	≥1.5	1415	1463	[19]
Haouzi <i>et al.</i> (2009)	LH + 2 (31)	LH + 7 (31)	≥2	945	67	[16]
Mirkin <i>et al.</i> (2004)	LH + 8 (5)	hCG + 9 Atg (5)	≥1.19	6	6	[25]
	LH + 8 (5)	hCG + 9 Ag (3)	≥1.2	5	5	
Horcujadas <i>et al.</i> (2005)	LH + 7 (14)	hCG + 7 (5)	≥3	281	277	[24]
Simon <i>et al.</i> (2005)	LH + 7 (14)	hCG + 7 Atg standard dose (4)	≥2	22	69	[26]
	LH + 7 (14)	hCG + 7 Atg high dose (5)		88	24	
	LH + 7 (14)	hCG + 7 Ag (5)		22	100	
Horcujadas <i>et al.</i> (2008)	LH + 7 (5)	hCG + 7 (5)	–	69	73	[22]
	LH + 9 (5)	hCG + 9 (5)		0	0	
Liu <i>et al.</i> (2008)	LH + 7 (5)	hCG + 7 high serum E2 levels (4)	≥2	244	159	[23]
	LH + 7 (5)	hCG + 7 low serum E2 levels (4)		5	2	

This table compares the number of genes significantly modulated between the LH + 2/4 and LH + 7/9 sample groups from five studies, as well as between LH + 7/9 days and hCG + 7/9 days sample groups from five other studies using the same approach.

Ag: Agonist; Atg: Antagonist; ESP: Early-secretory phase; hCG: Human chorionic gonadotropin; LH: Leutenizing hormone; MSP: Mid-secretory phase.

expression profiles associated with endometrial receptivity during COS (human chorionic gonadotropin [hCG] + 7/9) were closer to those in natural cycles (LH + 7/8), either under GnRH antagonist or agonist treatments [25,26]. However, the number of genes significantly modulated under COS in comparison with natural cycles was very different between these two studies (112 genes under GnRH antagonist treatment in Simon's study vs 12 genes in Mirkin's study; 122 genes under GnRH agonist treatment in the Simon's study vs six genes in Mirkin's study). Finally, Simon's study concluded that endometrial development during cycles using GnRH antagonists was found to be histologically more similar to those from natural cycles than endometrium exposed to GnRH agonists [26]. Horcujadas's study revealed that endometrial development was hampered during COS cycles, as judged by the number of genes significantly modulated under COS cycles in comparison with natural cycles (558 genes), showing gene expression levels more similar to those in a nonreceptive endometrium [24]. More precisely, they have demonstrated the existence of a 2-day delay in the activation/repression of two clusters composed of 218 and 133 genes, respectively, on day hCG + 7 versus LH + 7,

affecting the endometrial receptivity process [22]. More recently, it has been reported that impairment of endometrial receptivity was due to a high concentration of steroids resulting from COS [23,27].

“...it has been reported that impairment of endometrial receptivity was due to a high concentration of steroids resulting from controlled ovarian hyperstimulation...”

Therefore, the real challenge is to perform COS treatment modifications to achieve a receptive endometrium that morphologically and functionally resembles the natural receptive endometrium, which will subsequently lead to improved success rates in IVF.

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